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Hydrogen Peroxide Inhibits Giant Cell Tumor and Osteoblast Metabolism In Vitro

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This study investigates the efficacy of using hydrogen peroxide as adjuvant therapy after extended local curettage for benign giant cell tumors of bone. Hydrogen peroxide is used clinically as a chemical adjuvant for removal of residual tumor cells, presumably by effervescent cleansing with minimal damage to surrounding soft tissue and bone cells. This investigation examined the effects of hydrogen peroxide on giant cell tumor cells and osteoblasts grown in culture. Fresh fragments of histologically confirmed giant cell tumor tissue (six patients) and trabecular bone (one patient) were excised. Cells obtained from the fragments were grown in culture. Confluent cell cultures were exposed to saline (control) or hydrogen peroxide (0.1–1000 mm) for 2 minutes, and incubation was continued for 12, 24, or 48 hours without hydrogen peroxide. Protein content, deoxyribonucleic acid content, tartrate resistant acid phosphatase activity, and alkaline phosphatase activity were measured in the cell layers. The medium from the final 12 hours of each incubation period was used to evaluate lactate production. Cell lysis or death occurred after exposing giant cell tumor cells and osteoblasts to 100 mm and 30 mm hydrogen peroxide, respectively, concentrations substantially lower than the 3% (880 mm) hydrogen peroxide commonly used clinically. These results support the theory of using a minimal concentration of hydrogen peroxide as a chemical adjuvant in the surgical treatment of giant cell tumors of bone.

Giant cell tumors of bone account for approximately 5% of all primary benign bone tumors.[7,14,17] They are relatively common lesions in adults, occurring mainly in the long bones, with a lower incidence in the vertebrae and the pelvis. Historically, the treatment of giant cell tumors has involved a surgical excision with wide margins when possible; however, more recent studies support the use of intralesional curettage, with or without various adjuvant therapies.[3–5,7,8,12,14,18,26–31] External beam radiation therapy, liquid nitrogen, alcohol and phenol, electrocauterization, and hydrogen peroxide (H_2O_2) have been used as local adjuvant therapies to treat giant cell tumors in various anatomic locations[3–5,7,8,12,14,16,18,19,26–31,35]; as a result, reported local recurrence rates have diminished to 15% to 40%.

The consensus is that extended local curettage combined with local adjuvant therapy is sufficient treatment for most benign giant cell tumors of long bones (Table 1);

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Exhibit M

however, the efficacy of any of the adjuvants has never been studied in a randomized fashion. Although some adjuvant therapies theoretically sterilize the surface of the tumor cavity (electrocautery, pH, H_2O_2), others may extend the interosseous surgical margin (cementation, cryosurgery). Most surgeons recognize that prevention of local recurrence primarily is dependent on their ability to perform an adequate extended curettage of the involved bone.[5,8,30] Using the combination of a large cortical window, a motorized burr, and a pulsatile lavage system, modern mechanical debridement should rid the host bone of all visible tumor. The objective of adjuvant therapy is to cleanse the involved bone cavity and surrounding soft tissue of microscopic tumor contamination; this can be achieved in various ways.

Johnston[19] first reported the use of H_2O_2 as a local chemical adjuvant for giant cell tumors. In a followup series of 38 patients treated with curettage, H_2O_2, and polymethylmethacrylate, the recurrence rate was approximately 8%, which is lower than the 17% to 45% rate for curettage and polymethylmethacrylate alone.[5] Series reporting the use of a cytotoxic adjuvant in addition to polymethylmethacrylate have among the lowest reported local recurrence rates. Although these clinical studies suggest a trend for reduced local recurrence rates with the use of cytotoxic agents, no reports quantifying the effects of chemical adjuvants on giant cell tumor cells are available.

This study evaluates the efficacy of H_2O_2 as a chemical adjuvant and provides more sound scientific support for use of H_2O_2. The effects of H_2O_2 on cultured giant cell tumor cells and normal human osteoblasts were investigated. The results provide in vitro support that H_2O_2 at low concentrations causes lysis and death of giant cell tumor cells.

MATERIALS AND METHODS

Cell Isolation and Culture

Giant Cell Tumor Cells

Fresh fragments of histologically confirmed giant cell tumor tissue were collected from six consenting patients at the time of excision (Table 2). Tumor fragments were placed into giant cell tumor

TABLE 1. Selected Literature Summary of Adjuvants for Giant Cell Tumor Excision

Author, Year	Cases	Adjuvant(s)	Local Recurrence Rates (%)	Side Effects
Ellis, 1949[12]	60	Radiation orthovoltage	20–94	7–25% late sarcoma
Bell et al, 1983[3]	15	Supervoltage	0	No sarcoma
Miller et al, 1990[30]	280	Curettage/BG	45	
McDonald et al, 1986[29]	112	Curettage/BG	34	
Campanacci et al, 1987[7]	16	Curettage/phenol/alcohol	13	
Eckardt and Grogan, 1986[11]	260	Curettage/phenol/alcohol	25–50	
Marcove et al, 1973[27]	25	Curettage/LN/BG	25	30% fracture, 30% infection
Miller et al, 1990;[30] Capanna et al, 1990[8]	20	Curettage/LN/BG	20	25% fracture
Marcove, 1982[26]	100	Curettage/LN/BG	8	
Miller et al, 1990;[30] Capanna et al, 1990[8]	33	Curettage/phenol/PMMA	3	
Miller et al, 1990;[30] Capanna et al, 1990[8]	187	Curettage/PMMA	17	7% arthritis
Johnston, 1987[19]	22	Curettage/H_2O_2/PMMA	7	
Bini et al, 1995[5]	38	Curettage/H_2O_2/PMMA	8	

BG = bone graft; LN = liquid nitrogen; PMMA = polymethylmethacrylate; H_2O_2 = hydrogen peroxide.

TABLE 2. Characteristics of Giant Cell Tumor Tissue

Patient Number	Donor Age (years)/Gender	Site of Tumor	Radiologic Grade	Histologic Grade	Days in Culture*
1	35/M	Distal radius	3	II/III	4
2	22/F	Proximal femur	3	II/III	5
3	40/M	Proximal tibia	3	II/III	5
4	32/M	Proximal fibula	2	II/III	0
5	29/F	Distal femur	3	II/III	7
6	36/M	Proximal femur	3	II/III	5

*Number of days giant cells were visible microscopically in culture.

cell growth medium consisting of Dulbecco's modified Eagle's medium (Sigma Chemical Company, St. Louis, MO) containing 10% Nu-Serum IV (Collaborative Research, Bedford, MA), 25 mm Hepes, 35 μg/ml glycine, 100 μg/ml ascorbic acid, 40 ng/ml vitamin B_{12}, 2 μg/ml *p*-aminobenzoic acid, 200 ng/ml biotin, and 100 U/ml-100 μg/ml-250 ng/ml penicillin-streptomycin-fungizone (pH 7.4).[33] The tumor fragments were processed immediately for cell isolation.

Each of the giant cell tumor tissue fragments was minced into 1 to 3 mm pieces with a scalpel. Cells were isolated from the tumor pieces by sequential digestion using a purified collagenase (375 units/ml, Type VII, Sigma) and protease (7.5 units/ml, Sigma) mixture. Details of the cell isolation procedure have been published previously.[33] Isolated cells were plated at a density of 2.5×10^5 cells/cm^2 in 12-well, plastic culture plates (Costar, Cambridge, MA). The cells were cultured in giant cell tumor cell growth medium to confluence (5–7 days) in a humid, 37° C incubator.

Osteoblasts

Normal trabecular bone was obtained from a consenting patient during a heterotopic ossification excision. A modification of the Robey and Termine method[36] was used to obtain osteoblasts. Trabecular bone fragments were minced into 2 to 3 mm pieces with a bone rongeur and enzymatically washed using purified collagenase (375 units/ml, Type VII, Sigma) to remove unwanted surface cells. The fragments were transferred into 25 cm^2 flasks (Costar) containing a modified phenol red and calcium free osteoblast growth medium comprised of 1:1 Ham's F12:Dulbecco's modified Eagle's medium (Sigma) containing 20% fetal bovine serum (Sigma); 0.1% insulin-transferrin-selenium (Collaborative Biomedical Products); plus glutamine, vitamins, and antibiotics and antimycotic as described for preparation of the giant cell tumor cell growth medium. Osteoblasts were allowed to migrate from the fragments and reached confluence after being cultured for 8 weeks. The human osteoblast cultures used in this study have been shown to contain high alkaline phosphatase activity, produce Type I collagen, increase cyclic adenosine monophosphate content in response to parathyroid hormone challenge, and produce osteocalcin when incubated in the presence of 1,25 dihydroxycholecalciferol.[21] The confluent cells were released using the purified collagenase and protease mixture described and were subcultured at a plating density of 6.25×10^4 cells/cm^2 in 12-well, plastic culture plates. The subcultured osteoblasts were grown in osteoblast growth medium to confluence (5 days) in a humid, 37° C incubator.

Histologic and Microscopic Analyses

The giant cell tumor curettage specimens were fixed in formalin and stained with hematoxylin and eosin using routine procedures. All giant cell tumor specimens were evaluated by an experienced musculoskeletal pathologist using light microscopic study. Each lesion was graded Type II/III according to the system of Jaffe et al[17] (Table 2) and each revealed histopathologic findings typical of a benign giant cell tumor, including prominent multinucleated giant cells whose nuclei were markedly similar to the nuclei of the surrounding mononuclear stromal cells.[17] In addition, one of the giant cell tumor specimens was stained histochemically for tartrate resistant acid phosphatase activity (an enzyme characteristic of osteoclasts) according to the method of Gruber et al[15] (Fig 1).

An inverted phase contrast microscope was used for daily observation of the giant cell tumor cells and osteoblasts during culture, and for photomicrography.

Acute Exposure to H_2O_2

After the medium was removed from the wells, the confluent cell layers were rinsed once with saline (0.9% NaCl) and exposed to either 1 ml of saline (control) or 1 ml of H_2O_2 (0.1–1000 mm in saline for giant cell tumor cells; 1–1000 mm in saline for osteoblasts) for 2 minutes. These ranges were chosen to include the commonly used clinical concentration of 880 mm H_2O_2 (3.0%). The 2 minutes simulated a common surgical exposure time. At the end of this exposure period, the solutions were removed and the cell layers were rinsed three times with 2 ml of saline. This was followed by incubation in respective growth medium for 12, 24, or 48 hours (giant cell tumor cells) or for 24 or 48 hours (osteoblasts). The cell layers, to which 1 ml of 1 m NaCl containing 0.1% Triton-X 100 and 0.01% trypsin (Sigma) was added, and the medium from the final 12 hours of each incubation period were stored at –20° C for subsequent analysis.

Analytic Methods

Cell layer alkaline phosphatase activity (an enzyme activity characteristic of osteoblasts) at pH 9.8[24] and tartrate resistant acid phosphatase activity at pH 5.0[1] were determined colorimetrically using *p*-nitrophenylphosphate as substrate. The enzymatic activity is expressed in units, where one unit represents 1 µmol of substrate hydrolyzed per hour at 37° C. Lactic acid released to the medium (an index of energy metabolism via glycolysis) was measured enzymatically using the protocol of Lundholm et al.[25] A protein assay kit (Bio-Rad Laboratories, Inc., Hercules, CA) was used to evaluate protein content.[6] The cell layers were frozen three times and thawed, followed by digestion with Proteinase K (5 mg/ml, Sigma) overnight in a 60° C shaker bath. Total deoxyribonucleic acid (DNA) content (a measure of cell number) was determined using Hoechst 33258 fluorescent dye[23] (Sigma) or PicoGreen (Molecular Probes, Inc, Eugene, OR) fluorescent dye.

Statistical Analysis

A one-way analysis of variance followed by the Tukey-Kramer (honestly significant difference) multiple comparison analysis was performed on the data. Except where noted, each group consisted of six wells. Individual group standard deviations were used to construct the 95% confidence intervals. The data are presented as the mean and 95% confidence interval with $p < 0.05$ considered significant.

RESULTS

Giant Cell Tumor Cells

Figure 2 depicts giant cell tumor cells cultured for 4 days. Although variations were observed in the giant cell tumor anatomic loca-

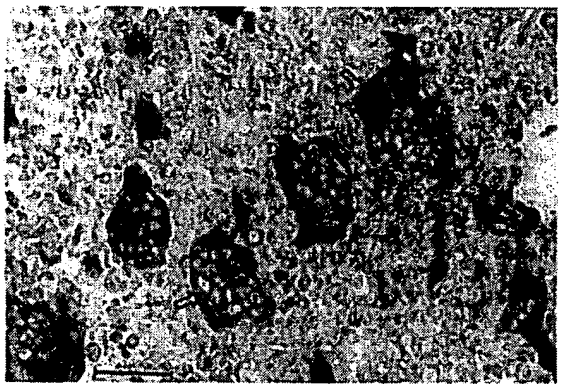

Fig 1. Giant cell tumor tissue histochemically stained for tartrate resistant acid phosphatase activity. Note stained, multinucleated giant cells (open arrows). Bar = 50 µm.

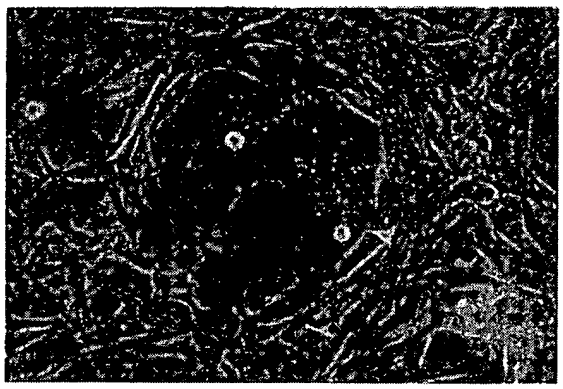

Fig 2. Cultures of giant cell tumor cells. Note multinucleated giant cell (arrow head) and stromal cells (arrows). Phase contrast. Bar = 50 µm.

tions, radiologic and histologic grades, and number of days the giant cells persisted in culture (from 0 to 7 days), the interassay results from each of the cultured giant cell tumors remained consistent among the patients. Immediately after being exposed to H_2O_2, the cell layers were observed microscopically. No differences in the cell layers were seen in the 0.1 to 30 mm H_2O_2 exposure groups when compared with their saline controls (Figs 3A–B); however, the cells that were exposed to 100 mm H_2O_2 appeared to effervesce slightly (Fig 3C). In addition, the bubbles that occurred when cells were exposed to 1000 mm H_2O_2 were visible macroscopically. In fact, some of the cells immediately began to detach from the culture surface at this concentration (Fig 3D).

Similarly, DNA content (a measure of cell number) was not affected until the giant cell tumor cells were exposed to 100 mm H_2O_2 (Fig 4A). At 12 hours after the 2-minute exposure, cell number declined 59% compared with the control; 24 hours after exposure, DNA content declined 83%; and, at 48 hours, cell number was near 0. Likewise, 12 hours after exposure to 1000 mm H_2O_2, cell number was essentially 0.

These DNA data are supported by the results obtained for lactate production (an index of glycolysis). Lactate production was not affected by H_2O_2 until 24 hours after ex-

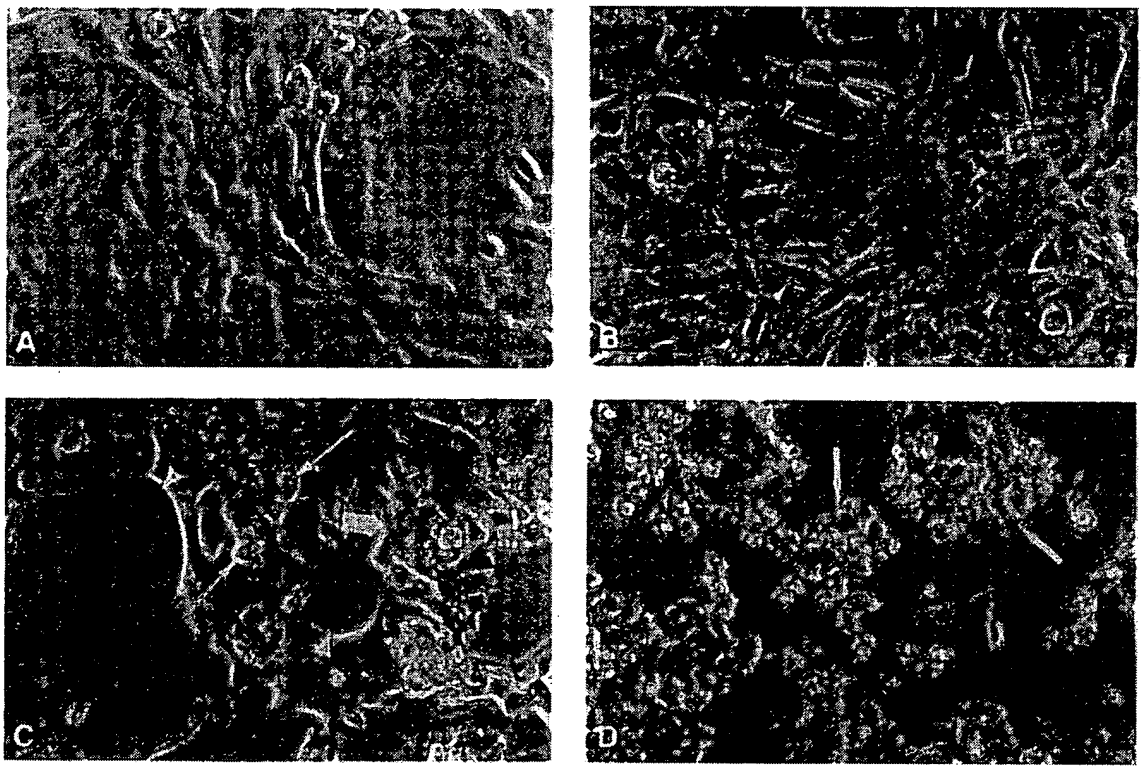

Fig 3A–D. Giant cell tumor cell cultures immediately after exposure to (A) saline (control), (B) 30 mm H_2O_2, (C) 100 mm H_2O_2, or (D) 1000 mm H_2O_2. Cells exposed to 0.1 to 30 mm H_2O_2 appeared to be similar morphologically to those exposed to saline even though biochemical differences were measured. The cells that were exposed to 100 mm H_2O_2 rounded up and partially detached from the culture surfaces, whereas the cells that were exposed to 1000 mm H_2O_2 completely detached and floated. Note multinucleated giant cell (A, arrow head), stromal cells (A, arrows; B, arrowheads; C, thin arrows), a partially detached group of stromal cells (C, thick arrow), and completely detached groups of stromal cells (D, arrows). Phase contrast. Bar = 50 μm.

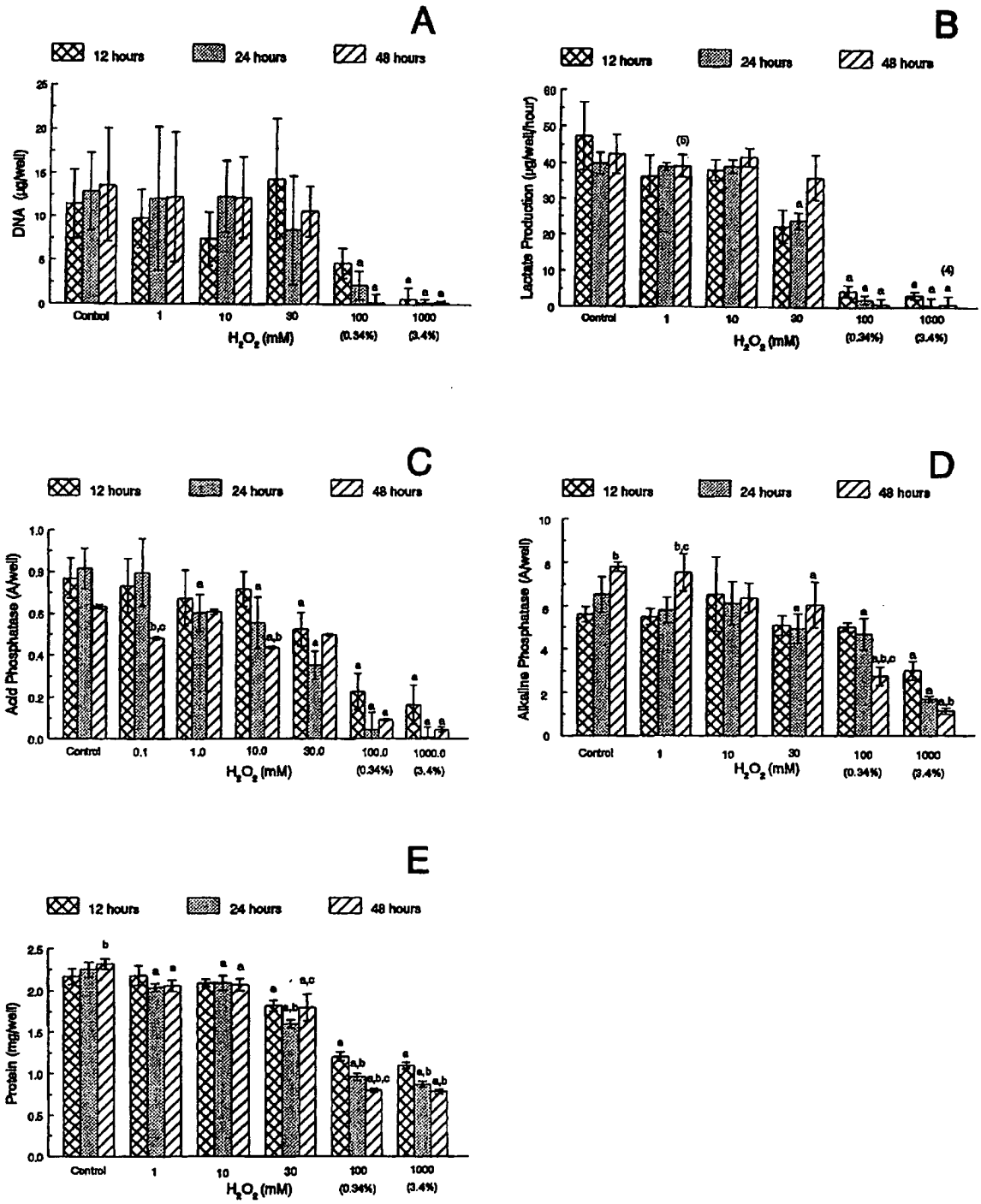

Fig 4A–E. Effects of hydrogen peroxide (H_2O_2) on giant cell tumor cells. Cells were exposed to saline (control) or H_2O_2 (0.1–1000 mm) for 2 minutes; (A) DNA content, (B) lactate production, (C) tartrate resistant acid phosphatase activity, (D) alkaline phosphatase activity, and (E) protein content were determined 12, 24, and 48 hours later. Each bar represents the mean of six wells, except where noted in parentheses. Vertical lines represent the 95% confidence intervals. $^{a}p < 0.05$, compared with the respective control at the same time; $^{b}p < 0.05$ compared with the same concentration of H_2O_2 at 12 hours; $^{c}p < 0.05$ compared with the same concentration of H_2O_2 at 24 hours.

posure to 30 mm H_2O_2 (Fig 4B); however, at 12 hours after exposure to 100 and 1000 mm H_2O_2, and thereafter, lactate production was reduced 90% or greater when compared with controls.

Histologic localization of tartrate resistant acid phosphatase in the multinucleated giant cells of the giant cell tumor tissue suggests that these are osteoclastlike cells (Fig 1). The inhibitory effects of H_2O_2 on biochemically measured tartrate resistant acid phosphatase in cultured giant cell tumor cells were seen at much lower concentrations than those affecting DNA content and lactate production (Fig 4C). Activity of tartrate resistant acid phosphatase was decreased 24% 48 hours after exposure to 0.1 mm H_2O_2. As with DNA and lactate production, tartrate resistant acid phosphatase was diminished substantially at 100 and 1000 mm H_2O_2 when compared with respective controls.

Activity of alkaline phosphatase (marker of osteoblastlike cells) in the cell layers was approximately 10-fold higher than tartrate resistant acid phosphatase (Fig 4D). A concentration related decline of alkaline phosphatase began at 30 mm H_2O_2. More importantly, alkaline phosphatase declined in a pattern similar to that of DNA at 100 and 1000 mm H_2O_2, but not nearly as much. When compared with respective controls, alkaline phosphatase was unchanged at 12 hours, and had declined 28% at 24 hours, and 64% at 48 hours after exposure to 100 mm H_2O_2. This decline was more evident at the 1000 mm exposure to H_2O_2: at 12 hours, it was 46%; at 24 hours, 74%; and at 48 hours, 85%.

Protein content (also influenced by cell number) in the cell layers began a slight, but significant ($p < 0.05$), 9% decline 24 hours after exposure to 1 mm H_2O_2 and continued to decline in a fashion similar to that of alkaline phosphatase for the duration of the experiment (Fig 4E). At high H_2O_2 concentrations (100 and 1000 mm), protein content and alkaline phosphatase activity in the cell layers failed to show the degree of inhibition seen in DNA, lactic acid, and tartrate resistant acid phosphatase.

Osteoblasts

Figure 5 depicts osteoblasts cultured for 5 days. As with the giant cell tumor cells, the osteoblasts were observed microscopically immediately after being exposed to H_2O_2. No differences in the cell layers were observed in the 1 and 10 mm H_2O_2 exposure groups when compared with the saline controls; however, the osteoblasts, which were more sensitive to H_2O_2 than were the giant cell tumor cells, appeared to effervesce slightly when exposed to 30 and 100 mm H_2O_2. Like the giant cell tumor cell cultures, the osteoblast cultures bubbled when exposed to 1000 mm H_2O_2. In addition, some of the osteoblasts immediately began to detach from the culture surface at this concentration.

These observations support the DNA results, which show that cell number is not significantly affected until the osteoblasts are exposed to 30 mm H_2O_2, when cell number declines to essentially 0 (Fig 6A); however, such a rapid decline in the giant cell tumor cell numbers was not observed until they were exposed to 100 mm H_2O_2.

Similarly, lactate production in the osteoblasts was not affected until 48 hours after exposure to 10 mm H_2O_2, when it decreased 83% (Fig 6B). At 30 mm H_2O_2, all glycolytic activity ceased. By comparison, glycolytic activity in the osteoblasts had ceased when gly-

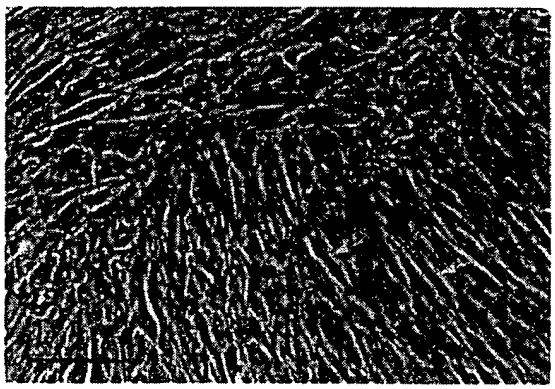

Fig 5. Cultures of normal human osteoblasts. Note osteoblasts (arrows). Phase contrast. Bar = 50 μm.

colytic activity in the giant cell tumor cells first was affected by exposure to H_2O_2.

The presence of tartrate resistant acid phosphatase was minimal in the osteoblasts; however, it decreased 40% 24 hours after exposure to 30 mm H_2O_2 (Fig 6C). In contrast, activity of tartrate resistant acid phosphatase in osteoclastlike cells was diminished at a concentration more than one order of magnitude lower (1 mm H_2O_2 at 24 hours) when the giant cell tumor cells were exposed to H_2O_2 (Fig 4C).

The alkaline phosphatase in the osteoblasts began to decline 48 hours after exposure to 10 mm H_2O_2 (Fig 6D). At 24 hours after exposure to 30 mm H_2O_2, alkaline phosphatase declined 22%; after exposure to 100 mm H_2O_2, 45%; and after exposure to 1000 mm H_2O_2, 57%. Activity of alkaline phosphatase declined 10% 48 hours after exposure to 10 mm H_2O_2; 23% after 30 mm H_2O_2; 34% after 100 mm H_2O_2; and 62%, after 1000 mm H_2O_2. Despite the alkaline phosphatase in the osteoblasts not being affected as severely as that in the giant cell tumor cells, the alkaline phosphatase in the osteoblasts never recovered from H_2O_2 treatment.

As with the giant cell tumor cells, protein content in the osteoblasts experienced a slight, but significant ($p < 0.05$), 18% decrease 24 hours after exposure to 1 mm H_2O_2; however, unlike the giant cell tumor cells, protein content of the osteoblasts continued a steady decline thereafter (Fig 6E). When compared with their respective controls 24 hours after exposure to 1 to 1000 mm H_2O_2, protein content decreased 18% to 46%, respectively. At 48 hours after exposure to 1 to 1000 mm H_2O_2, protein content decreased 10% to 85%, respectively.

DISCUSSION

The current in vitro study shows that H_2O_2 is a suitable adjuvant for surface sterilization after excision of giant cell tumors. Despite differences in the number of days the giant cells persisted in culture (from 0–7 days), the cells responded to H_2O_2 similarly. Although biochemical changes may have been found in the giant cell tumor cells exposed to saline or to 0.1 to 30 mm H_2O_2, no morphologic changes were seen. However, damage to the giant cell tumor cells exposed to 100 and 1000 mm H_2O_2 was instant, substantial, and microscopically visible.

The cell layers effervesced immediately on exposure to 100 and 1000 mm H_2O_2. This reaction may be enhanced by the enzyme catalase, which is found in bone and may help to ameliorate the toxic effects of H_2O_2 by converting it to water and oxygen.[10,34,39] Although anecdotal references suggest that caution be taken to prevent the development of gas emboli from oxygen bubbles,[38] no clinical reports of emboli from using H_2O_2 as adjuvant treatment of giant cell tumors have been documented.

Physiologically, H_2O_2 is capable of damage at concentrations that are quite low. Inflammatory cells (neutrophils and macrophages) can produce nanomole quantities of H_2O_2 that damage cell membranes, inactivate enzymes, and damage DNA.[13] It also has been shown that synthesis of proteoglycans in bovine[2] and mouse[37] articular cartilage is inhibited by low concentrations of H_2O_2 (0.05–0.1 mm). Low concentrations of H_2O_2 can cause cell death in human or epithelial cell cultures[22] and inhibit glucose metabolism and collagen synthesis in chick embryo tibias and osteoblast cultures.[20,32] Although the clinical use of 3% H_2O_2 (880 mm H_2O_2) was not a problem in the series of Bini et al[5] and the 21-year series of Johnston,[19] the current study establishes evidence that a concentration nearly one order of magnitude lower remains effective in causing giant cell tumor death in vitro. Although some damage to surrounding tissue is expected, there have been no reported negative clinical effects of exposure to H_2O_2. However, this in vitro study shows that there may be substantial damage to surrounding normal osteoblasts when they are exposed to 30 mm H_2O_2 (0.1%). Thus, damage to surrounding bone and soft tissue

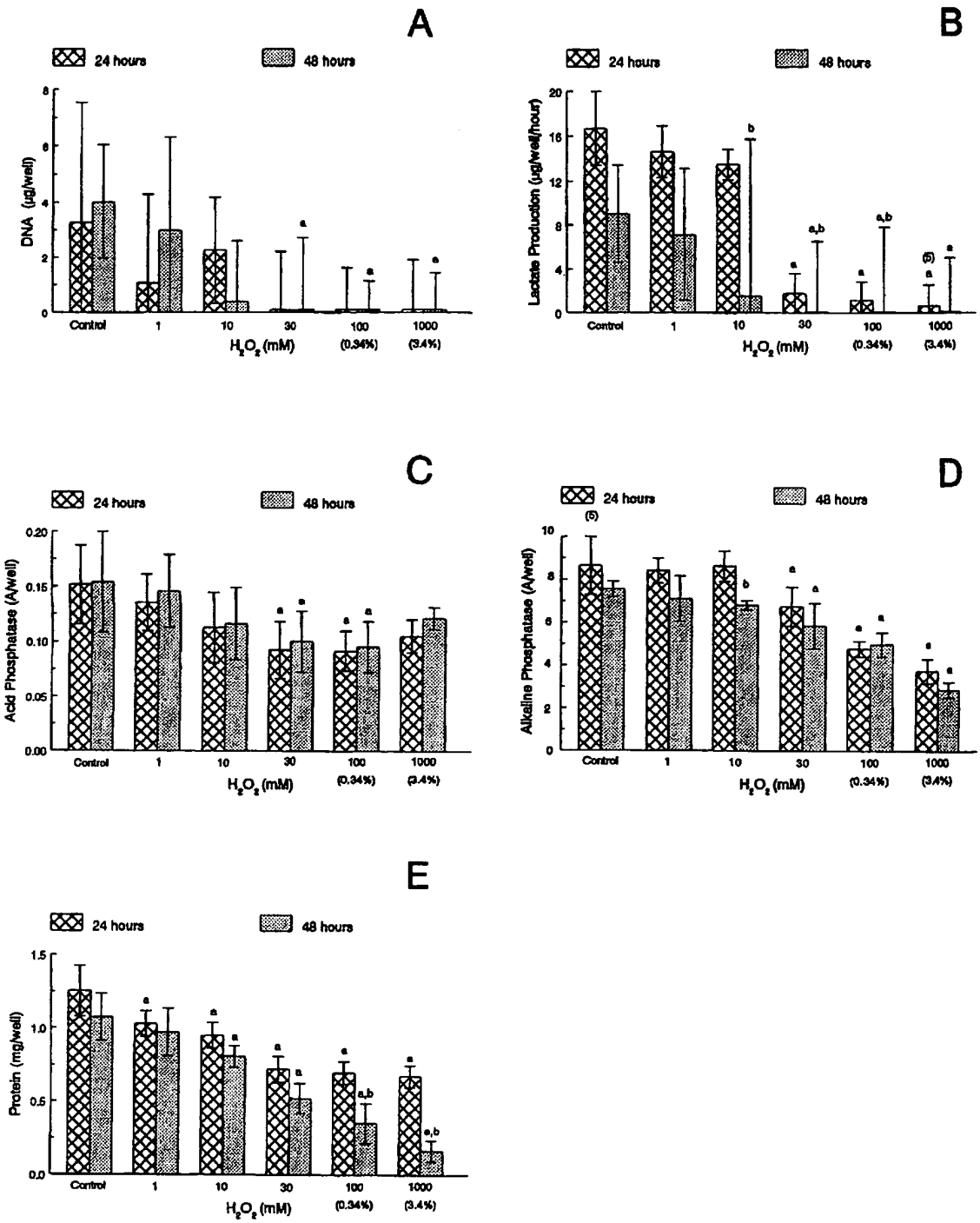

Fig 6A–E. Effects of hydrogen peroxide (H_2O_2) on osteoblasts. Cells were exposed to saline (control) or H_2O_2 (1–1000 mm) for 2 minutes; (A) DNA content, (B) lactate production, (C) tartrate resistant acid phosphatase activity, (D) alkaline phosphatase activity, and (E) protein content were determined 24 and 48 hours later. Each bar represents the mean of six wells, except where noted in parentheses. Vertical lines represent the 95% confidence intervals. $^{a}p < 0.05$ compared with the respective control at the same time; $^{b}p < 0.05$ compared with the same concentration of H_2O_2 at 24 hours.

could be substantial, even at low concentrations of H_2O_2, but this type of damage was not studied.

Inhibition of lactate production by H_2O_2 in giant cell tumor cells correlates well with its effect on DNA (cell number). Energy metabolism via glycolysis appears to have been depressed significantly 24 hours after cells were exposed to 30 mm H_2O_2; however, the cells recovered by 48 hours. The dramatic decline in glycolytic activity after exposure of the cells to 100 and 1000 mm H_2O_2 may occur because the cells have been lysed or killed by the H_2O_2. The damaging effects of H_2O_2 on lactate production (glycolysis) also are evident in the osteoblast data. Although glycolysis in the cell layers is not affected significantly until 48 hours after exposure to 10 mm H_2O_2, the lactate production results confirm the DNA evidence that cells were lysed or killed when exposed to H_2O_2. In addition, the high variability of the means of the treated groups probably is the result of immediate lysis or death of the osteoblasts when exposed to H_2O_2.

The decline of tartrate resistant acid phosphatase positive osteoclastlike cells depicts an expected trend of decreasing giant cell tumor cell quantity and quality as a result of being exposed to H_2O_2. This result may reflect a loss of the large, multinucleated giant cells that are known to have high tartrate resistant acid phosphatase activity.[9,18,40] In contrast, low tartrate resistant acid phosphatase values in the osteoblast cell layers suggest that there are few osteoclastlike cells present in the osteoblast population that was isolated.

The increase in alkaline phosphatase activity and protein content in the giant cell tumor cell control cultures at 48 hours is not accompanied by an increase in cell number (DNA content). In addition, unlike the tartrate resistant acid phosphatase, alkaline phosphatase and protein content are never inhibited by H_2O_2 to the extent that DNA and lactate are. The detection of alkaline phosphatase in the giant cell tumor cell cultures suggests the presence of an osteoblastlike or fibroblastlike cell in the tumor stroma. It is possible that a portion of the alkaline phosphatase, which is a membrane bound enzyme, is retained in the cell layer matrix after giant cell tumor ablation. Similarly, the cell protein data suggest that there is protein in the matrix that remains even after the giant cell tumor cells are destroyed by exposure to H_2O_2, which also supports this theory.

The current study presents in vitro evidence supporting the use of H_2O_2 as an appropriate chemical adjuvant to achieve ablation of giant cell tumor cells that may contaminate surrounding bone or soft tissue after intralesional or marginal giant cell tumor excision. Although this model does not replicate the true clinical situation, H_2O_2 is highly efficacious in killing giant cell tumor cells in vitro at a concentration that is nearly an order of magnitude lower than the commonly used clinical concentration. If a lower concentration of H_2O_2 were used, it could help to minimize damage to surrounding normal osteoblasts, soft tissue, and bone matrix in adjacent bone. This basic science study supports the theory that H_2O_2 adjuvant therapy for giant cell tumors may be a significant factor in achieving the reported 8% recurrence rate, which appears to be lower than that of many recent series using curettage and polymethylmethacrylate alone.[5]

Acknowledgments

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